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TECHNICAL NOTE

Application of cold flush preservation to in vitro microperfusion studies of kidney tubules

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The technique of microperfusion in vitro of isolated segments of the nephron, first described by Burg et al [1], has led to major advances in our understanding of nephron function. The technique has been applied to all segments of the nephron, many of which are inaccessible to other forms of study (see *Kidney Int* 22(5), 1982).

Two limiting factors that have influenced the application of this technique are: *(1)* the ease with which a tubule segment can be dissected from kidney tissue and *(2)* the viability of the tubule segment during perfusion, particularly when proximal segments are perfused. Many laboratories have limited their observations to New Zealand white rabbits, which possess kidneys that are more readily dissectable than those of other mammals; even so, the occurrence of readily dissectable kidneys varies considerably. Kidney tissue is customarily cooled for dissection to minimize the development of ischemic tubule damage [2]; nevertheless, it is common practice to use another rabbit if the dissection time exceeds 30 to 60 min.

We have recently made use of a technique that permits extension of the dissection time and provides several other practical advantages. The technique involves the application of a modified cold preservation procedure similar to that used in kidney transplantation. It arises from a new type of cold flush solution which consists of a phosphate buffer with added sucrose (PBSuc) shown to provide excellent preservation of tubule morphology in rat kidneys [3]. Our observations suggest that it also offers effective preservation of function as judged by measurements of fluid reabsorption rate (J_V), transmural potential difference (PD), leak of iothalamate from lumen to bath, and the responses to removal of intraluminal glucose and alanine.

Methods

Male, grade IV, specific-pathogen-free (S.P.F.) New Zealand white rabbits (Hackin and Churchill, Huntingdon, United Kingdom), weighing 1.5 to 2.0 kg, were anaesthetized with pentobarbitone (35 mg/kg i.v.). One of two protocols was then followed. In one group of animals, the left kidney was removed rapidly and treated in the conventional manner [4] for in vitro microperfusion. In the other group, a laparotomy was performed and the viscera reflected to expose the left kidney and renal artery. The artery was cannulated with polyvinyl 02 tubing and flushed from a raised reservoir at approximately 1.5 ml/min with cool (17°C) isotonic PBSuc 140 (NaH_2PO_4 13, Na_2HPO_4 56, Sucrose 140 mmoles/liter). Blanching of the kidney occurred immediately. After 20 ml PBSuc140 had passed through the vasculature, the kidney was carefully removed and stored in flush solution at 4°C for up to 48 hr. The kidney was then sliced and dissected in cold flush solution.

Midcortical segments of proximal convoluted tubule were dissected from both groups of kidneys and set up for microperfusion on a conventional apparatus (J. White, Bradbury Park, Maryland, USA) as described previously [4]. Tubules were perfused by gravity at 11 to 12 nl/min with physiological saline (NaCl 114, $NaHCO_3$ 25, K_2HPO_4 2.5, $MgSO_4$ 1.2, $CaCl_2$ 2.0, glucose 5.5, alanine 6.0, Na lactate 4.0, Na_3 citrate 1.0 mmole/liter) and bathed in the same solution, which also contained 6 g/dl purified dialyzed bovine serum albumin (Sigma Chemical Co., St. Louis, Missouri, USA). J_V was derived from measurements of ^{125}I-iothalamate (Amersham International, Amersham, United Kingdom) in perfused and collected fluid; transmural PD (lumen negative) was measured by standard techniques. The leak of iothalamate from bath to lumen was measured in all tubules.

Two series of experiments were carried out. In Series I, four groups of tubules were studied: in the control group, seven tubules were dissected from non-flushed (NF) kidneys with minimal delay; three groups of tubules were dissected from flushed kidneys that had been cold-stored for less than 4 hr (F, $N = 9$), for approximately 24 hr (F_{24}, $N = 9$), and for approximately 48 hr (F_{48}, $N = 6$). Tubules were perfused at 37°C and reached steady state within 30 min. Data presented are the mean of three measurements made in the 15-min period 45 to 60 min after commencement of microperfusion at 37°C. The NF group was compared with each of the three experimental groups using Dunnet's test [5].

Series II experiments were carried out to ascertain whether the apical cotransport mechanisms for glucose-Na^+ and amino acid-Na^+ remained functional after 24 hr of cold preservation. Two groups of tubules (NF, $N = 6$; F_{24}, $N = 6$) were perfused and bathed with the solutions described above. After 30-min equilibration, measurements were made of transmural PD (not corrected for junction potential) and tubule outside and inside diameter. Measurements were repeated after the perfusate had been exchanged for one in which glucose and alanine were replaced isosmotically with NaCl, and then again in a final

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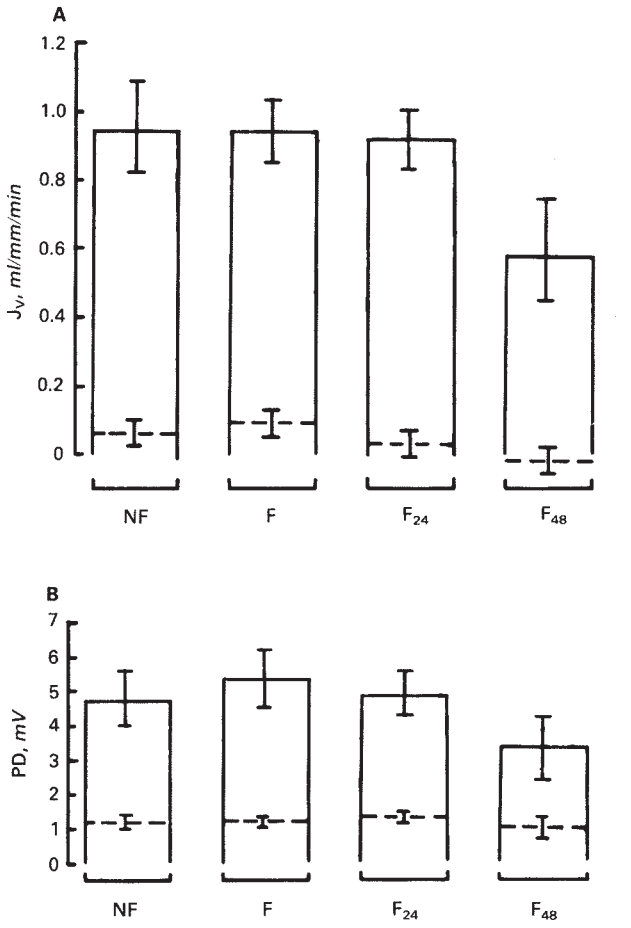

Fig. 1. *Mean ± SEM fluid reabsorption rate, J_V (a) and transmural PD (b) after 45 to 60 min of perfusion at 37°C (———) and during perfusion at 20°C (------).* For abbreviations, see **Methods.**

control period with the organics restored. Twenty-min equilibration was allowed after each perfusate change before measurements were made. Internal diameter (ID, to the apex of the brush border) and outside diameter (OD) were measured from photographs. Cell cross sectional area (CSA) was calculated from $\pi[(OD)^2/2 - (ID)^2/2]$. Data from both control periods were pooled and compared with that from the experimental period using a paired t test. In all cases, differences were regarded as significant when $P < 0.05$.

Results

Series I

Figure 1A and B show that after 45 min of perfusion at 37°C, groups NF, F, and F_{24} exhibited similar mean fluid reabsorption rates (J_V) and transmural PDs. Mean J_V and PD were lower in F_{48} tubules, the reduction in J_V compared with group NF was significant ($P < 0.05$). Iothalamate leak, expressed as a percentage of perfusion rate for this period, was: NF, 2.6 ± 0.5, F, 1.7 ± 0.3, F_{24}, 2.9 ± 0.6, F_{48}, 2.6 ± 0.6. There were no significant differences among the groups. Following 37°C perfusion, the temperature of the bath was reduced to 20°C to test for active transport; in all groups, both J_V and PD fell to low levels (Figs. 1A and B).

Series II

Removal of glucose and alanine from the perfusate in F_{24} tubules caused a reduction in mean transmural PD from 4.42 ± 0.22 mV to 1.03 ± 0.23 mV ($P < 0.001$) and a reduction in CSA from 1612 ± 78 μm^2 to 1282 ± 93 μm^2 ($P < 0.002$). In the NF tubules, transmural PD fell from 4.42 ± 0.22 to 1.99 ± 0.33 mV ($P < 0.005$) and CSA fell from 633 ± 49 μm^2 to 1338 ± 25 μm^2 ($P < 0.001$).

Discussion

The data clearly show that midcortical proximal convoluted tubule segments dissected from kidneys flushed with PBSuc140 solution and stored for up to 24 hr displayed J_Vs and PDs similar to those of tubules dissected immediately from non-flushed kidneys. The low bath leaks of iothalamate confirmed that tubule integrity was maintained. While performing this study, we also looked at the effect of 4 to 24 hr cold storage on four tubules each from non-flushed kidneys and from kidneys flushed with physiological saline. All of these tubules had extremely high bath leaks of iothalamate and, therefore, unmeasurable J_V. Preservation of function by the PBSuc flush, therefore, appears to be related to the nature of the solution rather than to the effects of clearing blood from the kidney.

It has previously been reported that the apical cell membrane glucose-Na^+ and amino acid-Na^+ cotransport mechanisms contribute significantly to the lumen negative PD of the proximal convoluted tubule and give the cells their characteristically swollen appearance [6]. In Series II, we found that removal of glucose and alanine from the perfusate significantly reduced transmural PD and CSA in both NF and F_{24} tubules. The observations on NF tubules thus confirm previous findings of Burg et al [6], while the similar observation on F_{24} tubules indicate that the apical sodium-dependent carrier-mediated systems for glucose and alanine remain functional after flush with PBSuc140 and 24 hr cold storage.

The protective action of the PBSuc140 solution may not be solely due to sucrose, as discussed by Coffey and Andrews [3]. Brazy et al [7] have shown the increased requirement for phosphate of the microperfused proximal convoluted tubule. The combination of an effectively impermeant solute, sucrose, with a high concentration of phosphate in the flush solution may be important both to prevent cell swelling and maintain intracellular phosphate prior to rewarming the tubule.

There are considerable benefits to be gained from the use of PBSuc140 flushed kidneys for microperfusion. Tubule dissection is facilitated because there is no longer a time constraint; in the present experiments, we found retrospectively that we had dissected longer segments of proximal tubule from flushed kidneys (F, 1.2 ± 0.1 mm; F_{24}, 1.4 ± 0.13 mm; F_{48}, 1.35 ± 0.26 mm) than from untreated kidneys (NF, 1.0 ± 0.07 mm). The use of a single kidney for several perfusion rigs in use on the same experimental day reduces the number of rabbits required, facilitates comparison of tubules from different parts of the same kidney, and allows optimum use of more readily dissectable kidneys. Difficulty in dissection is commonly related to the incidence of renal interstitial fibrosis [8] caused by the endemic microsporidian parasite, *Encephalitizoon (Nosema) cuniculi,* found in up to 95% of rabbits in accredited (non-S.P.F.) breeding colonies in the United Kingdom [9]. In this study, we

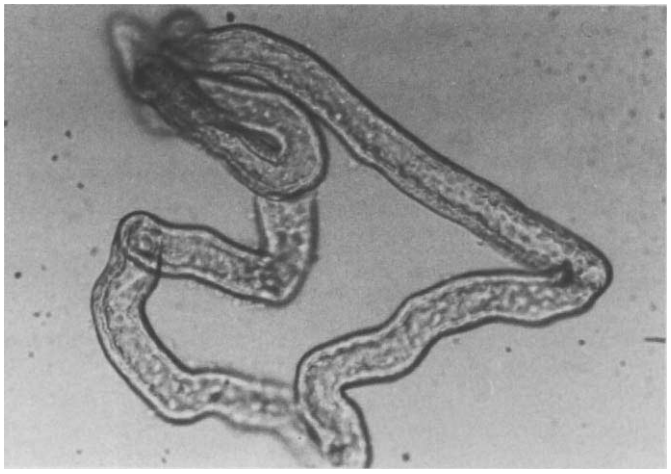

Fig. 2. *Tubule from group F with open lumen prior to mounting on pipettes.* Mean outside diameter is 37 μm.

used exclusively Grade IV specific-pathogen-free rabbits with their low incidence of parasite infection [10]. In every case we were able to dissect a viable length of proximal tubule without difficulty.

There was a notable difference in the appearance of tubules from non-flushed and flushed kidneys immediately prior to being mounted upon the pipettes. The lumina of tubules of group NF were closed whereas those in tubules from flushed kidneys were open (Fig. 2), due to the presence of the impermeant sucrose in the lumen. Insertion of the fine perfusion pipette into a closed tubule and subsequent opening of the lumen by the perfusion of fluid can damage the cells, particularly if the rate of opening is too rapid [11]. The possibility of such damage is eliminated in the flush-preserved tubule with its open lumen. It is not yet clear whether all cell transport components are equally well preserved after 24 hr cold preservation and account should be taken of this when designing experiments. We are studying other aspects of cold preservation at present.

Acknowledgments

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